ON THE PRESENCE OF TWO TYPES OF SUBUNIT IN SOYBEAN AGGLUTININ

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1. Introduction

Soybean Agglutinin (SBA), a glycoprotein lectin (mol. wt. 120 000) is comprised of four subunits of 30 000 daltons each [1]. The lectin interacts specifically with *N*-acetyl-D-galactosamine and D-galactose [2] and possesses two saccharide binding sites per 120 000 daltons [1]. Although the number of binding sites is half the number of subunits, no differences could be previously detected between the subunits [1].

Recently it has been demonstrated that discontinuous polyacrylamide gel electrophoresis at alkaline pH, in the presence of urea or sodium dodecyl sulfate (SDS), has a high resolving power in separating similar polypeptide chains. With this technique, differences have been found between the subunits of tubulin [3,4] and of the lectin from *Dolichos biflorus* [5,6], proteins that were believed to be comprised of identical subunits.

We have now reinvestigated the subunit structure of SBA using the above technique and found that the lectin is composed of two types of subunit. These were separated by ion exchange chromatography in the presence of urea and were found to differ in their charge.

2. Materials and methods

Soybean agglutinin was purified by affinity chromatography on Sepharose-*N*-ε-aminocaproyl-β-D-galactopyranosylamine [7]. The lectin was radioactively labeled by oxidation of its carbohydrate side chain with sodium periodate, followed by reduction with sodium [^{3}H] borohydride [8].

Polyacrylamide gel electrophoresis was performed in the following systems: (a) In sodium phosphate buffer, pH 7.0, containing 0.1% SDS (BDH, specially pure) [9]. (b) In a discontinuous anionic, pH 9.7 glycine system in the presence of 8 M cyanate free urea (Schwartz/Mann, Ultrapure) and 0.1% SDS [5,10]. (c) In an alkaline discontinuous boratesulfate system in the presence of 0.1% SDS [11]. The gels were stained for protein with Coomassie brilliant blue R-250 [9] and for glycoprotein with fuchsin-sulfite [12]. For the determination of radioactivity in gels, they were frozen on dry ice, immediately after electrophoresis, and sliced into sections 1mm thick. Each slice was incubated in 1 ml of 30% (v/v) H_2O_2 at 90°C for 1 hr prior to addition of 10 ml of Bray's liquid and counting in a Packard Tri-Carb scintillation spectrometer.

Ion exchange chromatography was performed at room temperature on a column (1 × 40 cm) of DEAE-cellulose (Whatman DE-52, microgranular, preswollen) in the presence of 8 M cyanate-free urea buffered with 0.04 M Tris–HCl pH 7.3 [6]. Protein in the eluted fractions was estimated spectrophotometrically at 280 nm.

Amino acid analysis was performed as previously described [1]. NH_2-Terminal amino acid determination was according to the dansyl chloride method of Gray [13] in 0.1 M sodium bicarbonate -8 M urea, pH 8.5 [1].

For sequence analysis, SBA (6 mg, 50 nmoles) was dissolved in *N,N*-dimethylbenzylamine and the sequencer (Beckman model 890C) was operated accor-

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ding to programme DMBA. The phenylthiohydantoin derivatives were identified by gas-liquid chromatography after silylation as well as on the amino acid analyzer after hydrolysis with hydroiodic acid (48% v/v in water) for 20 hr at 130°C.

3. Results and discussion

Electrophoresis of soybean agglutinin at pH 7.0 in the presence of 0.1% SDS [9] affords, as previously found [1], a single protein band (fig.1, gel 1) with a migration corresponding to a mol. wt. of 30 000. However, discontinuous gel electrophoresis at alkaline pH

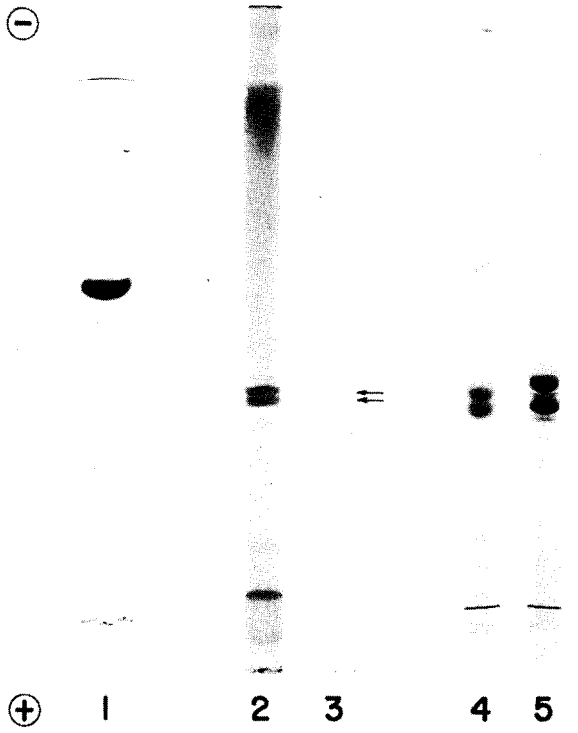

Fig.1. Polyacrylamide gel electrophoresis of soybean agglutinin on: 1, continuous pH 7.0 gel containing 0.1% SDS [9]; 2, discontinuous, alkaline, borate-sulfate gel containing 0.1% SDS [11]; 3, same as 2 but stained for glycoprotein [12]; 4, discontinuous pH 9.7 glycine gel containing 8 M urea and 0.1% SDS [5,10]; 5, same conditions as 4 only the lectin from *Dolichos biflorus* [5] was applied on the gel. Protein samples in the range 20–40 μg were used, at higher loads the separation into distinct bands (gels 2–4) was not efficient. Migration direction was from the top.

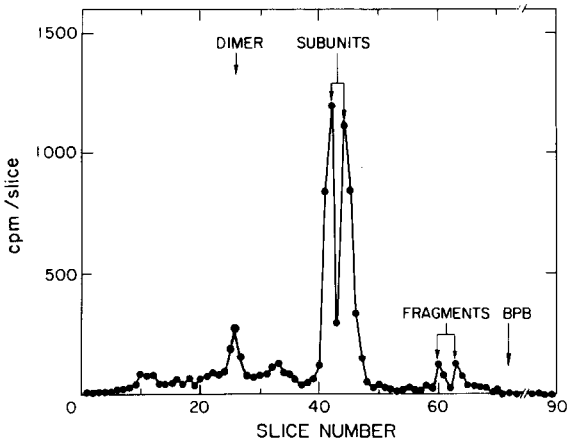

Fig.2. Discontinuous polyacrylamide gel electrophoresis of [^{3}H] soybean agglutinin (15 μg, 15 000 cpm) on alkaline borate sulfate gel in the presence of 0.1% SDS. BPB-Bromophenol blue. The migration was from left (cathode) to right (anode).

(>9.0) in the presence of 0.1% SDS [11] or in the presence of 8 M urea and 0.1% SDS [5], separates two protein bands (fig.1, gels 2 and 4, respectively). In the latter gels the upper band was designated subunit I and the lowersubunit II. For comparison the electrophoretic pattern of the lectin from *Dolichos biflorus*, where a similar phenomenon was observed [5,6] is also given (fig.1, gel 5).

The two types of SBA subunit stained for glycoprotein (fig.1, gel 3) which indicates that both contain carbohydrate. This was further demonstrated by electrophoresis of radioactively labeled SBA on the discontinuous alkaline borate-sulfate gels. As can be seen in fig.2, the dissociated lectin gives two major radioactive bands which seem to be labeled to the same extent, suggesting that the ratio between the subunits is 1:1.

The location of the slow moving band (slices 24–28) and of the fast moving bands (slices 59–62 and 63–66), correspond to those of the subunit dimer (mol. wt. 60 000) and of the subunit fragments, previously described by us [14]. The presence of only one band of subunit dimer suggests that the tetrameric lectin is comprised of two types of subunit that associate in the form, I_2 II_2 rather than being a mixture of two types of tetramer I_4 and II_4 which would yield two types of subunit dimer upon partial dissociation.

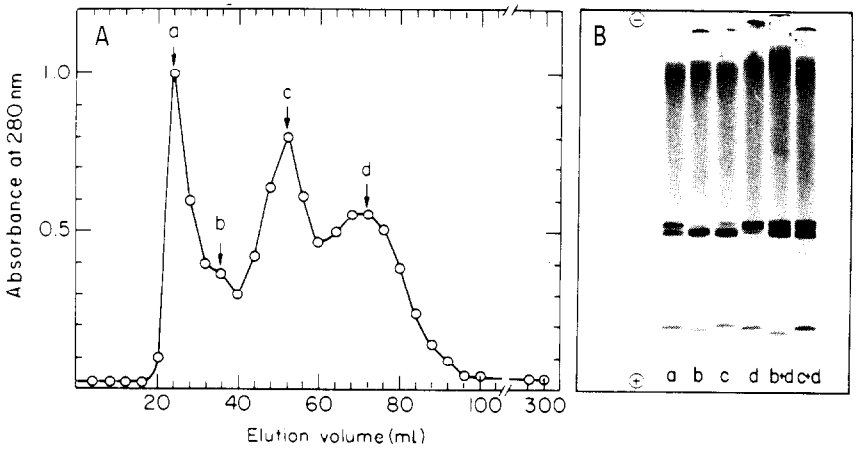

Fig.3. (A) Ion exchange chromatography of soybean agglutinin on DEAF-cellulose in 8 M urea pH 7.3. Soybean agglutinin (20 mg) was dissolved in 1 ml of 8 M urea in 0.04 M Tris-HCl pH 7.3 and applied on a column (1 × 40 cm) of DEAE-cellulose equilibrated in the same urea solution which was also used for elution of 2 ml fractions at a rate of 5 ml per hr. All operations were at 23°C. (B) Discontinuous polyacrylamide gel electrophoresis on alkaline borate-sulfate gels in the presence of 0.1% SDS [11] of soybean agglutinin fractions separated on DEAE-cellulose (fig.3,A). Samples of 30 μg protein were applied on each gel. In order to further demonstrate the separation of subunits by DEAE-cellulose, the recombined fractions b and d as well as c and d were also analysed by electrophoresis, here samples of 20 μg of each fraction were applied on the gels.

The two subunits stain with the same intensity for protein and for glycoprotein which also indicates that they are present in a 1:1 stoichiometry in the undissociated SBA.

Three protein peaks were eluted upon chromatography of SBA on DEAE-cellulose in the presence of 8 M urea (fig.3,A). The recovery of protein was 80–90% of that applied on the column and no additional protein could be eluted from the column by washing it with 1 M NaCl in 8 M urea.

Analysis of the protein fractions by discontinuous alkaline boratesulfate gel electrophoresis in the presence of 0.1% SDS [11] revealed that the first peak (fig.3,A peak a) contained the two subunits, the second peak (fig.3,A peak c) contained protein that co-electrophoresed with subunits II (compare with fig. 1, gels 2 and 4) and the third peak (fig.3,A peak d) contained protein which co-electrophoresed with subunit I. Upon rechromatography of peaks c or d under the same conditions as above, they emerged at the same position as before.

Attempts to renature the soybean agglutinin from the peaks separated by ion exchange chromatography were not successful, since upon dialysis of either peak or combinations of different peaks, the protein precipitated and could not be redissolved in buffers that did not contain either SDS or urea.

Attempts to fractionate SBA by ion exchange chromatography on DEAE-cellulose in the presence of 8 M urea at pH 9.5 were unsuccessful, since all the protein was adsorbed to the column and could be eluted, as a single peak, with a linear NaCl gradient (0 to 10 mM) in 8 M urea.

Amino acid analysis of peak c (subunit II) (fig.3,A) and peak d (subunit I) revealed a great similarity. The only significant differences were an excess of one lysine residue in subunit I and an excess of two aspartic acid (or asparagine) and one glutamic acid (or glutamine) in subunit II.

NH_2-Terminal amino acid analysis of the isolated subunits revealed that alanine was the NH_2-terminal amino acid in both subunits. Ten cycles in the sequencer gave a single amino acid at each step and the sequence found was: Ala–Glu–Thr–Val–Ser–Phe–Asp–Trp–Phe–Glu.

The different migration of the subunits on discontinuous alkaline pH gel electrophoresis in the presence of 0.1% SDS alone or with 8 M urea and the failure of continuous pH 7.0 gel electrophoresis in the presence of 0.1% SDS to separate the subunits, suggests

that they differ in their ratio of charge to frictional coefficient [11]. This is in accord with the results of our previous studies on SBA subunits [1] in which no difference in their molecular weight could be observed.

Acknowledgements

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